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NUTRITIONAL REQUIREMENTS OF LACTIC ACID BACTERIA

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II. Vitamin and amino acid requirements of *Streptococcus thermophilus* strains

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Nutritional requirements of lactic acid bacreria

The effects of certain inorganic and organic compounds on the growth of lactic acid bacteria were described in a previous paper from this laboratory[1]. One of the most interesting findings was that *Streptococcus thermophilus* strains require calcium for growth and another that the calcium requirements varied greatly with the composition of the growth medium. For instance, certain organic compounds (citric acid and oxalacetic acid) inhibited the growth of the strains in calcium-free media, but growth took place when 5—10 μg of calcium was added per millilitre of growth medium. A marked calcium requirement was observed with many strains even when the medium did not contain any of the growth-inhibiting compounds. According to these preliminary experiments calcium has a specific growth-promoting action, a fact that led to the plausible conclusion that calcium possesses some special biochemical function in the metabolism of certain strains of lactic acid bacteria. The nature of this function cannot be explained until we know what relationships exist between calcium and other nutritional components of the media. In the present study we have accordingly attempted to map out such relationships by investigating the requirements of *Str. thermophilus* strains for vitamins of the *B* complex, for purine and pyrimidine bases, for amino acids, and for some other components of the growth media both in the presence and absence of calcium.

Experimental

The cultures, their maintenance, and the preparation of inoculum cultures were described in the previous paper[1].

Compositions of basal media

The compositions of the basal media used are shown in Table 1. In the preparation of these media the procedure described in our previous study was followed[1].

General procedure

The experimental procedure was basically the same as in our previous work[1]. The growth of the lactic acid bacteria was followed turbidimetrically and the extent of growth recorded as galvanometer readings on a Klett-Summerson photoelectric colorimeter with a 590—660 mμ filter. The results in the tables are also galvanometer readings.

The experiments were carried out at least in duplicate. The general procedure in each experiment was to study the growth response to each compound both in the absence and presence of calcium under otherwise exactly identical growth conditions. In the experiments where it was present the concentration of calcium was 10 μg per millilitre of growth medium.

Results and Discussion

The effect of divers components of the basal media on the growth of six *Streptococcus thermophilus* strains was studied. Results of these experiments are shown in Table 2 b. The concentrations of the compounds investigated are given in Table 2 a, and are indicated by the numbers 1, 2, 3, 4, and 5 in Table 2 b. Number 1 indicates that the compound in question had not been added to the medium.

Table 2 b shows that of the investigated strains, Str 10, Str 12, and Str 74 did not grow in a medium containing no ascorbic acid or calcium, whereas Str 10 and Str 74 grew in a medium containing 10 μg Ca^{++}/ml. Addition of 5—20 mg of ascorbic acid to 100 ml of the latter medium led to a good growth of all strains. No correlation was found between the requirements of ascorbic acid and calcium in other experiments which will not, however, be reported here. In fact, the above strains also did not respond to ascorbic acid in most growth media.

All the tested strains grew well without Casamino acids (a mixture of amino acids). As the medium lacking Casamino acids contained only the amino acids L-glutamic acid, L-cystine, and DL-tryptophan, there is reason to suppose that the amino acid requirements of the tested *Str. thermophilus* strains are comparatively simple. Detailed experiments with amino acids are described below (from Table 4 onwards).

Solution *E*, which was used as buffer (and growth factor) in the media, was tested to find a suitable concentration. The organic anions in this solution (acetic acid, succinic acid, and β-glycerophosphoric acid) were discussed separately in the previous paper[1]. The present experiments show that solution *E* is essential for the growth of all tested *Str. thermophilus* strains. The volume added to our media (5 ml/100 ml) was in most cases slightly below the optimum, but as a larger volume of solution E was liable to cause complete growth inhibition, the volume added was not altered. The growth of strain 74 was inhibited when 10 ml of solution E was added to 100 ml of the medium (Table 2 b).

Tween 80 and oleic acid were present in the media used in the above experiments. Table 2 b shows that this emulsion was not necessary for, nor promoted the growth of the strains. Furthermore, the utilization of the emulsion by the strains may well have depended on the calcium content of the medium. Accordingly, Tween 80 and oleic acid were mostly omitted from the media used in the experiments described below, especially as they were likely to be disturbing factors in the investigation of the effect of calcium.

The purine and pyrimidine bases adenine, guanine, uracil, and xanthine were tested. None was essential to the strains and uracil and adenine were found to have an inhibiting effect in most cases. Other purine and pyrimidine bases present in most of the media used in the previous experiments were thymine, cytosine, and hypoxanthine; these were omitted because the strains tested did not correspond to their presence and were not studied in detail.

Table 3 shows the results of experiments on the effects of vitamins of the B complex.

Thiamine was not essential for the growth of any of the tested strains, but the growth of most strains was weaker in the thiamine-free medium than in the control medium. Thiamine is thus a growth-stimulating factor.

Riboflavin was essential for the growth of all tested strains. The weak growth obtained in the riboflavin-free medium (Table 3), is not significant because the strains had been transferred from a medium containing a high concentration of riboflavin, and the growth was most likely a consequence of this. The growth of most strains was slightly better in the medium containing calcium.

Nicotinic acid was essential for the growth of most strains and highly stimulated the growth of the others.

Biotin must be considered essential for the growth of all strains. It is true that a weak growth was observed with a few strains also in the absence of biotin, but this observation can hardly be considered significant.

Pyridoxal phosphate was not essential for the growth of any of the strains but, as can be seen from Table 3, it stimulated them all considerably.

p-Aminobenzoic acid was not essential for the growth of any of the strains tested.

Pantothenic acid was essential for the growth of all tested strains, independent of whether the medium contained calcium or not.

In this connection it may be mentioned that, as *folic acid*, *pyridoxin*, and *cobalamin* had no effect in the earlier experiments, these compounds were omitted from the medium. However, they were first tested together in the same medium to confirm the earlier observations, but no clear effect was observed (Table 3).

Tables 4—10 show the results of experiments on the amino acid requirements of twenty *Str. thermophilus* strains. These experiments were carried out by omitting one amino acid at a time from the group of amino acids added to the medium.

The growth medium (Sc 2) used in the experiments for which the results are shown in Table 4 differed slightly from the media (variations of ScB) used in the other experiments; the vitamin concentrations were only half of those used in medium ScB and the ammonium salt was omitted. However, the lowered vitamin concentration was not likely to affect the results in any marked degree nor could the omission of ammonium salt have any essential effect if one considers the results of control experiments in which a fairly large addition of several amino acids (18) was made. A factor that really did affect the results was the composition of the different amino acid mixtures used in these experiments. These compositions are given in the tables.

The results shown in Tables 4—6 are summarized in Table 7 to show results obtained with each amino acid separately, although different media were used in these experiments. The results are all affected by several factors, of which the most important are the presence or absence of other amino acids and calcium in the medium. Accordingly, a positive sign appearing in Table 7 does not necessarily mean that the amino acid in question is essential, nor does a negative sign mean that the amino acid always inhibits the growth of a certain strain. For instance, DL-isoleucine which was mostly found to inhibit growth in the experiments recorded in Table 7 proved highly stimulating to the same strains when another medium was employed.

L-Glutamic acid is essential for the growth of most strains listed in the tables. The calcium content of the medium was not observed to influence the glutamic acid requirements of any of these strains. Neither was any inhibiting effect of this amino acid observed even at the highest concentration used, 40 mg/100 ml.

L-Cystine is also essential for the growth of all strains tested. In a few cases the cystine requirements were observed to decrease slightly when calcium was added to the medium. The concentration 5 mg/100 ml gave a good growth response.

DL-Tryptophan was essential for the growth of only one tested strain (Th) but highly stimulating to most of the strains. This effect was often weakened when calcium was added to the medium.

DL-Tryptophan was often used together with L-glutamic acid (20 mg/100 ml of each), L-cystine (5 mg/100 ml) and ammonium chloride (37 mg/100 ml) as a nitrogen source in medium ScB in which all the strains listed in these tables have been tested several times. The results described below were obtained with this »minimal» medium; these experiments are not presented in detail here.

Most strains grew fairly well in the »minimal» medium when it *contained calcium*. If by »maximal growth» we mean the growth in medium ScB containing 500 mg of Casamino acids per 100 ml, the growth in the »minimal» medium was 50 per cent of the »maximal» growth with the following strains: Str 11, Str 11R, Str 77, Str 100, Str 101, Th, Ths, and Kungsb. k.; Str 110 and Str 111 did not grow in the medium because they require L-histidine (cf. Table 10). Str 10 R, Str 12, and Str 40 grew very weakly (the growth was less than 30 per cent of the »maximal» growth) in this medium.

Some strains grew in the *calcium-free medium* ScB even when it contained only three amino acids (glutamic acid, cystine, and tryptophan); these were Str 10, Str 11, Str 11 R, Str 72, Str 74, Str 75, Str 77, Str 100, Str 101, Str 102, Str 103, Th, Ths, Hansenin termostreptokokki (in the Table = H.th. str.), Kungsb.k., and *Str. th*. 7952 (ATCC). In most cases the growth was 10—30 per cent of the »maximal» growth.

When *L-histidine* and *L-tyrosine* (5 mg/100 ml of each) were added to the medium, the growth of all these strains improved very much. The growth of the strains listed below exceeded 50 per cent (many even 80 per cent) of the »maximal» growth: Str 10, Str 11, Str 11 R, Str 12, Str 40, Str 74, Str 101, Str 103, Str 110, Th, H.th.str., and *Str. th*. 7952. The growth of the following strains was 30—50 per cent of »maximal» growth: Str 75, Str 100, Str 102, and Str 111. Only with two strains, Str 72 and Ths. was the growth between 10 and 30 per cent, and with one strain, Str 10 R, less than 10 per cent of the »maximal» growth.

The above experiments have demonstrated that only L-glutamic acid, L-cystine, DL-tryptophan, L-histidine, and L-tyrosine can be considered essential for the growth of all tested strains. A positive sign, and in uncertain cases the sign x, appears fairly often in Tables 7 and 10 for other amino acids (especially DL-valine, glycine, DL-threonine, and, in Table 10, DL-methionine).

No discrepancies have been observed in the numerous experiments with different amino acid mixtures and single amino acids in varying concentrations; all the differences actually found can be ascribed to antagonistic relationships between the various amino acids present in the media or to the effect of calcium, which proved to be an important factor (Tables 7 and 10). A corresponding phenomenon is mentioned by Meinke and Holland (1950)[2]. Our work on the antagonistic relationships between amino acids and on the effect of calcium is being continued.

DL-Valine and glycine stimulate the growth of most of the strains (when both +, x, and s signs are considered). However, with most strains this effect was observed to either disappear or change to inhibition when calcium was added to the medium. The effect of DL-tryptophan described above is similar in the few cases where any effect was observed, and also the effect of DL-threonine changed in a similar way when calcium was added. With DL-aspartic acid and DL-alanine, however, also an opposite effect of calcium was observed with several strains, although very often the result of the calcium addition was the same as above.

The results obtained are dependent on many experimental factors, as a comparison between Tables 7 and 10 will show. A critical examination is therefore not yet possible. All that can be said at this phase of the study can equally well be seen directly from the tables. Ultimately, a detailed analysis of these results is likely to lead to a clarification of the mechanism of action of calcium, to which object the experiments presented in this paper are only preliminary. Considering, however, the above findings, the solution may well be found in the effect of calcium on the amino acid metabolism.

Summary

In this study the effects of various components of a growth medium on the growth of *Streptococcus thermophilus* strains have been examined both in the presence and in the absence of calcium. Ascorbic acid was found to be a growth-stimulating compound, but its effect depended on the chemical composition of the basal medium. The calcium content of the medium did not, however, affect the results. The emulsion of oleic acid and Tween 80 slightly stimulated the growth of most strains; this effect was more marked in a medium containing calcium than in one free from calcium. Of the purine and pyrimidine bases only uracil was observed to have any marked effect on the growth, and that an inhibiting one. Riboflavin, biotin, and calcium pantothenate were essential for the growth of all tested strains. Nicotinic acid was essential for the growth of most strains and stimulated the growth of all. Also thiamine and vitamin B_6 proved to be stimulating factors, but no clear effect of p-aminobenzoic acid, folic acid, or cobalamin was observed. The amino acids L-glutamic acid and L-cystine (cysteine) were essential for the growth of all strains, L-histidine for the growth of two, and DL-tryptophan for the growth of one strain. The requirements of the above amino acids were not dependent on the calcium content of the medium. In a calcium-free medium DL-valine, glycine, DL-threonine, DL-methionine, and DL-isoleucine were essential for the growth of most strains, but in a medium containing calcium they mostly inhibited growth. Also the effects

of L-aspartic acid, L-leucine, DL-alanine, L-tyrosine, and DL-tryptophan on the growth of the tested strains were found to vary with the calcium content of the medium.

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Table 1. The compositions of the basal media (quantities per 100 ml).

Component	Quantity	E1	Sc1	Sc2	ScA	ScA1	ScA2	ScA3	ScB
Sucrose	mg	500	500	500	500	500	500	500	500
Lactose	»	500	500	500	500	500	500	500	500
Ascorbic acid	»	20	20	20	20	20	20	20	20
DL-Aspartic acid	»	10	—	—	—	—	—	—	—
L-Glutamic acid	»	5	5	5	5	5	5	5	
L-Cystine	»	5	5	5	5	5	5	5	
DL-Tryptophan	»	10	10	10	10	10	10	10	—
Casamino acids	»	500	500	500	500	500	500	500	—
Xanthine	»	1	1	—	1	1	1	—	—
Tween 80+oleic acid	ml	0.5	0.5	—	0.5	0.5	0.5	—	—
Solution D	»	5	5	5	5	5	5	5	5
Solution E	»	5	5	5	5	5	5	5	5
Thymine	μg	50	—	—	—	—	—	—	—
Cytosine	»	50	—	—	—	—	—	—	
Hypoxanthine	»	50	—	—	—	—	—	—	—
Adenine	mg	1	1	1	1	1	1	—	
Guanine	»	1	1	1	1	1	1	—	—
Uracil	»	1	1	—	—	—	—	—	—
Thiamine	μg	50	50	50	50	50	100	100	100
Riboflavin	»	50	50	50	50	50	100	100	100
Nicotinic acid	»	50	50	50	50	50	100	100	100
p-Aminobenzoic acid	»	50	50	50	50	50	100	100	100
Calcium pantothenate	»	50	50	50	—	—	—	50	50
Sodium pantothenate	»	—	—	—	50	50	50	—	—
Biotin	mμg	50	50	50	50	50	100	100	100
Pyridoxal phosphate	μg	0.2	—	—	5	50	100	100	100
Pyridoxamin phosphate	mμg	5	—	—	—	—	—	—	—
Pyridoxal	μg	10	—	—	—	—	—	—	—
Pyridoxine HCl	mg	2	2	2	2	—	—	—	—
Folic acid	mμg	500	500	500	500	—	—	—	—
Cobalamin	»	11	11	11	11	—	—	—	—

Table 1 continued.

Stock solutions:

Solution D: $FeSO_4 \cdot 7$ aq, 20 mg $MnSO_4 \cdot 4$ aq, 20 mg $MgSO_4 \cdot 7$ aq, 3 g KCl, 6 g in 500 ml	Solution E: Na-Glycero-2-phosphate, 23.4 g Succinic acid 14.8 g Na-acetate . trihydrate 34.0 g in 500 ml

Tween 80 + oleic acid: Tween 80 (4 g) + oleic acid (40 mg) in water (40 ml)

Table 2a.

The concentrations of the compounds listed in the Table 2 b and there indicated by the tube numbers 1, 2, 3, 4, and 5. The underlined figures give the concentrations added to the medium of Anderson and Elliker[3].

Abbreviation	Compound	Tube number 1	2	3	4	5	Concentration
Asc	Ascorbic acid	—	5	10	$\underline{20}$	40	mg/100 ml
Glu	L-Glutamic acid	—	$\underline{5}$	10	20	40	—»—
Asp	DL-Aspartic acid	—	5	$\underline{10}$	20	40	—»—
CaPa	Calcium pantothenate	—	25	$\underline{50}$	75	100	µg/100 ml
TwO	Tween 80 + oleic acid	—	250	$\underline{500}$	750	1000	µl/100 ml
E	Solution E	—	2½	$\underline{5}$	7½	10	ml/100 ml
Ade	Adenine	—	½	$\underline{1}$	1½	2	mg/100 ml
Gua	Guanine	—	½	$\underline{1}$	1½	2	—»—
Ura	Uracil	—	½	$\underline{1}$	1½	2	—»—
Xan	Xanthine	—	½	$\underline{1}$	1½	2	—»—
Cys	L-Cystine	—	2½	$\underline{5}$	7½	10	—»—
Try	DL-Tryptophan	—	5	$\underline{10}$	15	20	—»—
Cas	Casamino acids	—	250	$\underline{500}$	750	1000	—»—

Table 2b.

The effects, both in the absence and in the presence of calcium, of some components of the basal media E1, Sc1, and Sc2 on the growth of six *Streptococcus thermophilus* strains. The abbreviations used in the table and the tube number corresponding concentrations are given in Table 2 a. Incubation 48 h at 42°C. The extent of growth is expressed as Klett-scale units.

Strain	Compound	Without calcium, Tube number 1	2	3	4	5	With 10 µg Ca^{++}/ml, Tube number 1	2	3	4	5	Basal medium
Str 10	Asc	—	29	96	90	72	56	89	78	81	74	E1
	Glu	86	84	80	79	73						»
	Asp	91	90	82	81	80						»
	CaPa	—	78	85	87	81	—	116	120	125	125	Sc1
	TwO	86	82	82	85	80	85	108	92	99	90	»

Table 2b continued

Strain	Compound	Without calcium Tube number 1	2	3	4	5	With 10 μg Ca^{++}/ml Tube number 1	2	3	4	5	Basal medium
	E	—	48	83	90	110	4	63	89	105	109	Sc2
	Ade	67	90	76	66	—	88	86	85	90	90	»
	Gua	80	84	84	73	58	73	69	73	77	76	»
	Ura	99	79	63	35	4	104	88	74	52	28	Sc1
	Xan	93	93	96	102	101	108	98	114	110	109	»
	Cys	68	—	55	74	92	82	80	83	79	78	Sc2
	Try	25	—	78	70	—	72	88	88	72	88	»
	Cas	23	74	76	72	74	57	100	110	100	97	Sc1
Str 12	Asc	—	+	58	71	50	—	58	108	117	104	E1
	Glu	88	87	79	61	53						»
	Asp	92	53	54	51	43						
	CaPa	—	99	92	90	94	—	102	93	107	00	Sc1
	TwO	99	89	92	98	88	103	118	112	120	126	»
	E	5	60	86	66	71	5	100	35	—	66	Sc2
	Ade	102	89	89	89	72	95	94	89	84	84	»
	Gua	76	88	78	97	84	79	67	54	66	69	»
	Ura	109	95	55	37	1	120	114	78	50	33	Sc1
	Xan	—	130	115	93	92	119	119	119	116	117	»
	Cys	70	68	82	95	—	72	75	50	65	86	Sc2
	Try	59	89	87	88	85	77	57	77	83	81	»
	Cas	20	80	99	97	107	67	118	115	116	110	Sc1
Str 74	Asc	—	35	59	104	62	93	155	150	146	144	E1
	Glu	130	109	127	100	107						»
	Asp	152	143	127	138	130						»
	CaPa	—	75	137	152	150	14	158	173	170	162	Sc1
	TwO	145	158	159	171	148	145	165	170	165	161	»
	E	—	85	120	112	—	—	61	114	88	95	Sc2
	Ade	103	109	117	100	65	130	132	76	105	95	»
	Gua	125	128	127	115	102	125	106	91	83	105	»
	Ura	162	152	100	21	—	164	145	110	70	43	Sc1
	Xan	166	156	159	171	148	158	163	167	167	170	»
	Cys	100	120	120	122	80	131	107	139	125	101	Sc2
	Try	29	115	117	105	—	116	115	90	90	111	»
	Cas	30	145	146	142	150	65	160	160	160	160	Sc1
Str 77	Asc	52	63	107	108	110	117	126	113	131	118	E1
	Glu	97	98	113	109	102						»
	Asp	115	110	106	109	108						»
	CaPa	—	120	119	118	118	9	131	134	127	134	Sc1
	TwO	117	115	106	117	99	120	118	120	99	128	»
	E	—	60	197	115	131	—	75	99	120	122	Sc2

Table 2b continued

Strain	Compound	Without calcium					With 10 μg Ca^{++}/ml					Basal medium
		Tube number					Tube number					
		1	2	3	4	5	1	2	3	4	5	
	Ade	101	106	106	89	23	117	122	132	117	120	Sc2
	Gua	108	108	113	117	108	119	111	113	111	111	»
	Ura	118	107	88	55	21	117	123	99	79	44	Sc1
	Xan	121	88	113	112	107	129	136	132	124	137	»
	Cys	91	—	66	101	—	114	114	124	120	126	Sc2
	Try	87	107	107	106	105	86	118	117	117	116	»
	Cas	51	125	106	112	111	47	123	120	131	121	Sc1
Str 110	Asc	119	104	112	114	120	120	112	115	154	156	E1
	Glu	119	127	129	110							»
	Asp	139	128	129	114	117						»
	CaPa	—	143	146	149	152	—	174	172	175	169	Sc1
	TwO	135	139	140	140	138	130	165	165	159	160	»
	E	—	72	121	132	120	3	93	135	144	135	Sc2
	Ade	103	79	87	90	100	119	141	116	132	95	»
	Gua	111	111	111	122	116	118	121	126	123	139	»
	Ura	155	147	103	152	18	166	163	130	78	39	Sc1
	Xan	154	158	150	142	140	170	170	168	168	168	»
	Cys	109	105	117	78	101	107	85	138	132	132	Sc2
	Try	82	104	114	97	115	116	103	114	123	140	»
	Cas	18	142	143	133	138	18	157	167	177	167	Sc1
Th	Asc	22	32	63	78	77	91	88	168	153	138	E1
	Glu	77	73	86	106	62						»
	Asp	142	148	145	135	122						»
	CaPa	—	138	130	131	121	—	157	162	162	163	Sc1
	TwO	125	128	123	127	129	147	149	142	157	153	»
	E	—	79	94	108	108	9	88	118	134	145	Sc2
	Ade	102	85	99	56	60	105	119	115	112	108	»
	Gua	71	92	93	91	75	101	111	110	112	112	»
	Ura	113	105	78	51	17	137	139	99	71	35	Sc1
	Xan	139	132	133	135	156	162	166	158	153	173	»
	Cys	77	50	76	94	74	96	111	102	87	105	Sc2
	Try	17	75	90	82	66	24	105	112	122	122	»
	Cas	31	133	119	132	121	54	153	154	141	111	Sc1

Table 3

Vitamin requirements of twenty *Streptococcus thermophilus* strains. Medium ScA was used with one of its vitamins omitted in each experiment except for the control experiment where all vitamins were present. Medium VB1 is the ScA medium supplemented with folic acid (1 μg/100 ml), pyridoxine (4 mg/100 ml), and cobalamin (22 mμg/100 ml.) Incubation 42 h at 37°C.

Strain	Without calcium					With 10 μg Ca^{++}/ml				
	Thiamine	Riboflavin	Nicotinic acid	Biotin	Control	Thiamine	Riboflavin	Nicotinic acid	Biotin	Control
Str 10	92	7	9	3	116	112	106	35	11	117
	98	6	5	—	108	113	9	10	8	117
Str 10R	104	6	7	19	146	111	7	7	4	122
	96	5	7	6	115	112	7	9	36	105
Str 11	91	—	29	—	123	106	3	36	4	130
	94	—	36	—	120	102	3	33	3	132
Str 11R	90	4	8	—	96	106	8	9	6	95
	85	4	9	—	96	100	6	9	10	102
Str 12	102	—	10	—	123	118	3	14	4	116
	103	—	10	—	115	116	4	11	4	116
Str 40	54	—	3	—	70	81	—	6	6	96
	60	—	5	5	130	70	—	8	6	99
Str 72	82	7	12	4	106	129	10	14	9	149
	100	7	12	4	121	123	11	14	9	156
Str 74	129	6	8	—	162	138	11	15	7	148
	127	7	10	—	151	133	9	11	7	161
Str 75		8	33	6	101	116	8	64	37	138
	86	8	36	4	100	107	7	68	37	134
Str 77	101	6	26	—	129	116	9	31	—	130
	95	—	23	7	123	112	7	28	—	130
Str 100	65	9	18	5	68	65	14	16	5	85
	58	8	21	6	63	75	14	27	4	92
Str 101	66	9	17	—	70	75	15	19	34	96
	61	10	17	—	74	90	15	22	7	94
Str 102	62	6	13	7	77	83	14	21	4	94
	75	9	17	4	77	72	17	19	5	93
Str 103	60	9	10	6	67	62	9	17	4	91
	60	8	12	6	69	60	8	19	4	93
Str 110	122	6	25	4	147	147	112	32	4	164
	134	5	23	6	147	158	2	32	5	167
Str 111	125	9	40	9	142	161	4	34	7	157
	109	9	38	8	145	140	3	38	6	165
Th	96	8	4	—	140	118	2	4	4	163
	103	7	5	—	139	114	—	4	5	157
Ths	130	8	26	5	146	108	9	23	7	144
	160	8	24	6	150	111	9	28	5	146
H.th.str	54	6	17	28	78	57	3	16	3	92
	53	5	19	—	78	55	3	16	3	92
Kungsb.k.	137	—	31	9	170	131	4	57	10	159
	130	156	33	11	171	142	6	27	9	161

Table 3 continued

Strain	Without calcium					With 10 μg Ca^{++}/ml				
	Pyridoxal phosphate	p-Aminobenzoic acid	Ca-pantothenate	Control-	VB1	Pyridoxal phosphate	p-Aminobenzoic acid	Ca-pantothenate	Control	VB1
Str 10	61	111	2	101	103	61	111	121	110	108
	59	102	2	101	101	60	111	—	107	112
Str 10R	66	152	—	147	143	63	125	91	144	148
	44	117	—	138	144	66	121	—	127	110
Str 11	36	111	—	118	120	42	124	—	126	123
	34	96	—	123	121	44	120	2	110	120
Str 11R	56	86	—	95	91	58	79	—	91	87
	51	96	—	79	93	53	93	—	91	91
Str 12	35	121	—	119	123	23	124	—	115	122
	53	120	—	115	129	42	122	—	120	120
Str 40	36	88	—	84	83	40	83	—	104	126
	38	78	2	87	64	42	105	—	95	98
Str 72	61	99	4	121	101	83	138	2	140	147
	76	100	5	115	120	77	143	1	143	145
Str 74	59	124	—	142	153	70	127	—	147	145
	62	117	—	141	140	71	142	—	143	146
Str 75	47	106	—	115	115	39	110	—	119	121
	48	109	2	115	114	45	117	—	127	123
Str 77	54	119	—	120	130	57	178	—	122	125
	58	126	—	121	125	41	121	—	123	125
Str 100	42	81	—	76	75	47	87	—	84	85
	46	81	—	79	80	45	84	—	79	85
Str 101	51	77	—	79	80	58	76	—	85	84
	44	80	—	77	85	46	78	—	87	89
Str 102	49	95	—	79	85	65	93	—	102	96
	83	93	—	78	84	55	94	—	96	93
Str 103	40	70	2	70	72	43	59	—	74	76
	38	67	—	89	70	43	74	—	76	75
Str 110	78	110	—	142	142	69	122	—	144	157
	71	114	—	145	133	82	134	—	152	159
Str 111	85	111	—	142	140	80	128	—	151	149
	85	116	3	140	125	82	130	—	149	160
Th	48	110	—	138	109	46	127	—	137	145
	53	111	—	119	113	62	118	—	143	143
Ths	13	130	—	133	135	36	119	—	133	141
	46	134	—	137	140	50	119	—	138	142
H.th.str.	47	95	3	88	95	47	123	—	118	112
	44	93	—	90	94	49	114	—	113	122
Kungsb.k.	50	160	—	167	164	45	160	—	162	168
	48	150	3	158	165	43	155	—	165	168

Table 4

The effect of the omission of one amino acid on the growth of five *Streptococcus thermophilus* strains in medium Sc2 without either the uracil, Tween 80, oleic acid, or amino acids listed in Table 1. In the control experiments all amino acids listed in the table were present. Concentrations used: 5 mg of L-amino acids and 10 mg of DL-amino acids per 100 ml of growth medium. Incubation 45 h at 37°C.

	Strains of Streptococcus									
Amino acid omitted	Str 10	Str 12	Str 74	Str 110	Th	Str 10	Str 12	Str 74	Str 110	Th
	Without calcium					With 10 μg Ca^{++}/ml				
L-Glycine	—	—	—	—	—	61	51	55	86	32
	—	—	—	—	—	58	51	53	85	27
L-Alanine	50	34	34	55	—	44	35	42	72	35
	54	37	53	62	—	48	40	44	71	30
DL-Valine	—	—	—	—	—	40	26	32	62	22
	—	—	—	—	—	45	23	46	67	—
L-Leucine	37	30	35	50	30	38	31	44	68	35
	29	32	42	44	28	42	34	44	72	40
DL-Isoleucine	77	54	64	61	57	74	50	92	85	56
	46	50	54	65	60	74	50	89	87	51
DL-Threonine	—	—	—	—	—	62	49	57	84	32
	—	—	—	—	—	59	53	57	84	32
DL-Serine	53	46	43	79	21	46	29	36	75	28
	50	46	39	78	32	37	35	40	76	32
DL-Methionine	46	41	41	70	30	33	34	41	68	32
	46	44	40	64	28	39	35	39	70	32
L-Cystine	—	—	—	—	—	—	—	—	18	5
	—	—	—	—	—	—	—	—	—	17
DL-Phenylalanine	50	63	47	86	36	50	45	41	77	37
	56	54	47	79	34	52	37	42	84	37
L-Tyrosine	57	54	41	80	38	52	42	44	84	33
	49	54	55	85	34	53	39	44	83	37
DL-Tryptophan	51	38	36	41	—	45	34	42	53	—
	48	8	36	43	—	48	36	40	54	—
DL-Lycine	40	48	56	68	27	50	34	40	72	37
	54	46	45	71	30	44	36	34	72	40
L-Histidine	38	40	38	—	44	40	30	34	—	49
	38	39	38	—	34	38	25	31	—	31
L-Arginine	42	39	45	58	17	38	33	34	65	9
	39	42	47	64	11	36	28	37	64	9
DL-Aspartic acid	39	—	30	48	6	47	30	—	74	44
	35	33	—	40	23	44	31	46	72	42
L-Glutamic acid	4	—	—	42	—	28	—	—	57	—
	23	—	—	—	—	—	30	—	60	—
L-Proline	60	47	53	77	24	50	30	45	83	42
	48	56	59	77	34	54	—	45	81	39
Control	53	60	80	36	30	53	38	43	77	34

Table 5

The effect of the omission of one amino acid on the growth of five *Streptococcus thermophilus* strains in medium ScB containing 27 mg of ammonium chloride per 100 ml of medium. In the control experiment all the amino acids listed in the table were present. Test concentrations: 1 mg each of threonine, valine, isoleucine, leucine, and serine, 20 mg each of glutamic acid and tryptophan, and 5 mg each of the other amino acids per 100 ml of medium. Incubation 45 h at 37°C.

Amino acid omitted	Strains of *Streptococcus*									
	Str 10R	Str 11	Str 40	Str 111	Ths	Str 10R	Str 11	Str 40	Str 111	Ths
	Without calcium					With 10 μg Ca^{++}/ml				
DL-Threonine	60	67	51	80	7	87	74	60	68	71
	50	55	46	76	7	65	75	64	70	64
DL-Valine	12	76	2	57	91	57	110	45	61	82
	26	81	33	46	80	63	110	37	77	79
DL-Isoleucine	38	63	26	98	100	107	84	41	58	106
	55	63	36	82	113	90	84	38	78	98
L-Leucine	29	40	20	70	87	75	75	25	87	87
	43	47	29	62	93	86	82	39	86	108
DL-Serine	58	67	50	76	101	78	76	58	88	101
	47	62	52	71	93	77	79	66	85	104
DL-Aspartic acid	51	64	46	73	97	72	73	54	57	73
	45	65	43	70	95	69	65	54	76	90
DL-Alanine	54	58	50	66	93	57	75	57	70	80
	40	57	54	72	76	63	79	57	64	86
L-Cystine	9	11	—	14	15	12	9	4	8	5
	6	12	7	30	—	14	4	1	8	7
L-Tyrosine	59	64	50	74	93	76	85	49	88	94
	59	69	56	80	107	84	78	60	91	90
L-Proline	51	69	51	81	105	76	80	57	85	99
	58	59	52	91	95	70	75	58	90	87
L-Phenylalanine	57	62	46	81	92	69	74	69	76	75
	60	59	54	75	98	76	77	69	85	87
DL-Methionine	15	49	40	63	78	21	71	52	75	109
	12	59	51	63	100	26	63	75	71	125
L-Arginine	62	61	47	68	96	70	83	55	61	92
	59	65	52	68	105	72	73	55	64	110
Glycine	63	51	40	50	83	54	53	46	51	83
	56	47	35	51	80	50	49	43	50	82
L-Lycine	52	63	52	61	94	95	77	53	73	74
	55	56	46	71	77	70	71	49	70	95
L-Histidine	57	67	48	3	91	77	91	52	—	96
	57	59	48	—	82	83	82	60	—	111
L-Glutamic acid	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	63	—	—
DL-Tryptophan	54	60	48	79	93	96	77	50	86	98
	44	63	47	72	100	67	83	56	84	106
Control	45	59	47	69	92	86	72	62	88	100
	45	55	49	78	100	88	75	62	88	92

Table 6

The effect of the omission of one amino acid on the growth of ten *Streptococcus thermophilus* strains in ScB medium containing 27 mg of ammonium chloride, 20 mg of L-glutamic acid, 20 mg of DL-tryptophan, and 5 mg of L-cystine per 100 ml of growth medium. Other amino acids present in the control experiments are as given in the table. Test concentrations: 1 mg each of threonine, valine, and isoleucine, and 5 mg each of all other amino acids per 100 ml of growth medium. Incubation 48 h at 37°C.

	Amino acid omitted										
Strain	Thr	Val	Ileu	Asp	Ala	Gly	His	Tyr	Phe	Control	
Str 11R	34	—	43	22	18	18	53	32	47	46	
	28	—		34	19	21	51	31	48	48	
Str 72	45	—	54	23	28	18	48	28	43	42	
	39	—	55	22	40	10	35	29	24	42	
Str 75	—	—	43	10	+	—	+	11	18	+	
	—	—	34	—	+	—	+	15	+	+	
Str 77	27	—	57	30	28	+	27	44	43	25	
	25	—	61	26	+	+	30	53	36	44	
Str 100	28	—	42	41	30	27	27	36	37	38	Without
	36	—	37	39	25	22	31	32	36	35	calcium
Str 101	42	—	50	46	35	35	38	44	39	38	
	39	—	41	48	35	37	37	37	34	36	
Str 102	46	—	49	29	—	17	36	32	34	38	
	48	—	48	21	27	22	36	53	50	35	
Str 103	37	—	26	36	27	27	28	32	35	31	
	35	—	46	22	27	26	31	29	35	26	
H.th.str.	70	17	63	43	70	50	72	67	71	68	
	75	21	65	45	55	50	67	70	71	75	
Str th.ATCC	44	—	97	—	—	21	+	10	41	38	
7952	44	—	55	19		16	47	—	56	36	
Str 11R	60	—	86	54	42	51	62	69	62	62	
	64	—	87	56	35	39	67	59	65	59	
Str 72	70	—	97	63	76	70	76	70	65	73	
	69	—	87	67	70	61	63	71	64	79	
Str 75	35	—	85	19	21	53	92	55	100	100	
	29	—	80	35	22	22	33	51	82	52	
Str 77	38	—	77	48	23	50	80	66	75	61	
	47	—	76	46	31	36	80	64	95	86	
Str 100	81	—	111	78	72	66	76	75	70	62	With 10
	76	—	93	63	64	61	71	77	63	67	$\mu g\ Ca^{++}/ml$
Str 101	79	5	48	79	73	79	83	76	74	70	
	82	16	60	82	76	72	79	78	71	67	
Str 102	88	—	91	57	56	54	88	87	87	83	
	93	—	82	49	53	53	87	87	80	81	
Str 103	66	38	61	72	65	59	80	75	65	63	
	68	12	72	67	62	68	71	75	67	62	
H.th.str.	64	+	76	50	55	60	59	80	65	73	
	65	+	80	52	52	56	69	80	65	63	
Str th. ATCC	59	—	72	48	90	45	64	66	51	54	
7952		—	65	35	39	—	64	85	53	74	

Table 7

Summary of results presented in Tables 4, 5, and 6. The extent of growth is compared to growths obtained in the control experiments. Signs used in the table:

+ = no growth (or less than 10 Klett-scale readings) observed when the amino acid was omitted.

s = much weaker growth than in the control experiment.

x = the result obtained in the first tube was +, that in the second *s*.

o = the growth was about the same as in the control experiment.

— = the growth was much better than in the control.

The signs of the left indicate results from a medium without calcium, those on the right results from a medium containing 10 μg Ca^{++}/ml. The pair of signs oo has not been indicated.

Strain	Glu	Cys	Try	Val	Gly	Thr	His	Ala	Asp	Leu	Ileu	Tyr	Phe	Met	Arg	Pro	Ser
Str 10	x+	++		+o	+o	+—	so		so	so	——			os			
Str 12	+x	++	so	+s	+—	+o		so	xo	so	——	—o	—o			—x	
Str 74	++	++	so	+o	+—	+—	ss		xx		——					o—	
Str 100				+o	so			so			o—				os		
Str 110	++	+x	ss	so	+—	+—	++		so		o—	——	——	so		——	—o
Th	++	+s	++	+x	+o	+o		+o	so		——				ss		
Str th 7952				++	sx		xo	xs	xs		——	xo	—o				
Str 11	++	++		——	ss		o—			so	o—						
Str 11R				++	ss	so		ss	ss		o—	so					
Str 72				++	so				os		——	so	so				
Str 75				++	+o	+s		os	xs		——	—o					
Str 103				+s			o—				oo	o—					
Str 101				+x		o—	o—		—s		—s						
Str 102				++	ss	——		xs	ss		——						
T.th.str.				ss	ss			os	ss		so	o—					
Str 77				++	ss	os	o—	xs	os		—o	—o	o—				
Str 10R	++	+s		ss	os			os	os	so	o—		so				
Str 40	++	++		xs	os				os	ss	ss						
Str 11	++	s+		+s	ss	os	++	os	os		—s			os	os	—o	
Ths	++	s+		os	ss	+o		os			—o		os	—o			

Table 8

The effect of the omission of one amino acid or ammonium salt on the growth of eight *Streptococcus thermophilus* strains in medium ScB. In the control all amino acids mentioned in the table were present. Test concentrations: 27 mg of NH_4Cl, 20 mg each of L-glutamic acid and DL-tryptophan, 1 mg each of DL-valine and DL-threonine, and 5 mg each of the other amino acids per 100 ml of medium. Incubation 42 h at 37°C.

Strain	Omitted compound											
	NH_4Cl	L-Glu	L-Cys	DL-Try	DL-Val	Gly	DL-Thr	L-His	DL-Ala	DL-Asp	DL-Met	Control	
Str 10R	22	—	—	26	13	15	30	18	6	37	—	29	
	29	—	—	2	8	9	17	33	13	28	—	50	
Str 72	22	—	—	19	24	21	24	12	41	38	—	43	
	27	—	—	26	23	23	24	15	38	35	—	45	
Str 75	12	—	—	—	28	—	—	2	14	—	—	18	
	15	—	—	—	14	—	—	10	11	—	—	27	
Str 100	26	—	—	—	20	20	28	25	17	17	—	17	
	24	—	—	—	20	19	21	26	16	11	—	32	Without
Str 102	22	—	—	16	18	9	16	22	15	13	—	15	calcium
	27	—	—	—	9	11	8	20	14	—	—	14	
Str 110	31	—	—	31	22	19	—	—	14	4	—	26	
	33	—	—	20	21	32	—	—	7	20	—	42	
Str 111	28	—	—	23	18	26	21	—	25	18	—	42	
	31	—	—	25	18	16	23	—	28	23	—	42	
Ths	24	—	—	55	70	50	53	50	71	62	—	73	
	25	—	—	67	65	52	62	44	67	71	—	78	
Str 10R	22	—	—	71	47	17	46	56	63	67	10	66	
	22	—	—	62	42	54	75	53	74	84	10	66	
Str 72	25	—	—	63	7	45	45	14	79	56	81	20	
	27	—	—	81	38	45	36	35	85	60	52	21	
Str 75	12	—	—	35	3	—	—	—	15	28	30	—	
	12	—	—	25	11	—	—	—	27	—	34	—	
Str 100	29	—	—	47	33	42	33	32	43	62	31	42	
	29	—	—	41	42	43	41	34	41	73	45	31	With 10
Str 102	28	—	—	21	—	13	14	19	—	33	32	20	μg Ca^{++}/m
	24	—	—	34	—	28	17	22	21	42	33	10	
Str 110	48	—	—	35	46	50	—	—	39	46	24	28	
	51	—	—	33	47	50	—	—	22	49	35	19	
Str 111	41	—	—	39	41	31	22	—	41	50	29	42	
	43	—	—	44	36	34	22	—	46	44	49	46	
Ths	—	—	—	78	—	—	17	16	72	—	87	—	
	—	—	—	63	—	—	13	18	68	—	80	—	

Table 9

The effect of the omission of one amino acid or ammonium salt on the growth of ten *Streptococcus thermophilus* strains in medium ScB containing 20 mg of L-glutamic acid and 5 mg of L-histidine per 100 ml of growth medium. All the amino acids listed in the table were present in the control tubes. Test concentrations: 27 mg of NH_4Cl, 20 mg of DL-tryptophan, and 5 mg of each of the other amino acids per 100 ml of medium. Incubation 42 h at 37°C.

Strain	Omitted compound: NH_4Cl	L-Cys	DL-Try	DL-Val	Gly	DL-Thr	DL-Ala	DL-Asp	DL-Ileu	Control	
Str 10	31	—	18	—	10	18	10	24	30	32	
	24	—	20	—	10	23	11	19	29	32	
Str 11	24	—	23	—	2	21	4	14	—	22	
	22	—	23	—	2	21	7	20	—	32	
Str 12	—	—	—	—	—	—	—	—	—	19	
	—	—	—	—	—	—	—	7	—	18	
Str 74	22	—	8	—	—	16	2	10	5	23	
	22	—	35	—	—	16	6	13	3	17	
Str 75	—	—	—	—	—	—	—	—	—	—	Without
	—	—	—	—	—	—	—	—	—	—	calcium
Str 100	19	—	19	—	23	20	9	24	15	24	
	23	—	18	—	21	18	15	22	20	26	
Str 103	21	—	15	—	13	21	9	15	—	19	
	19	—	16	—	11	23	4	16	2	24	
Str 110	22	—	20	—	—	21	5	27	5	44	
	24	—	27	—	—	23	5	18	15	30	
Str th	4	—	—	—	—	—	2	—	—	—	
7952 (ATCC)	—	—	—	—	—	—	—	4	—	—	
Th	8	—	—	—	11	8	—	9	—	17	
	5	—	—	—	9	—	22	25	—	8	
Str 10	34	—	54	—	47	51	36	48	68	49	
	28	—	54	—	40	57	36	47	85	49	
Str 11	30	—	51	—	33	53	23	42	56	45	
	24	—	54	—	31	52	20	50	4	40	
Str 12	18	—	15	—	7	7	—	28	42	13	
	24	—	16	—	3	—	—	24	8	8	
Str 74	33	—	54	—	58	52	52	69	80	57	
	32	—	36	—	60	49	59	72	73	63	With 10 μg
Str 75	16	—	26	—	26	33	37	44	58	32	Ca^{++}/ml
	15	—	18	—	21	46	28	44	16	36	
Str 100	27	—	44	—	48	53	—	36	45	39	
	25	—	44	15	47	—	47	42	55	49	
Str 103	25	—	42	4	45	47	44	38	47	42	
	25	—	40	10	45	44	45	36	45	44	
Str 110	47	—	47	—	52	48	30	46	44	44	
	58	—	40	—	58	33	33	54	44	46	
Str th	19	—	17	—	18	24	19	20	5	24	
7952 (ATCC)	11	—	8	—	21	26	15	16	8	21	
Th	17	—	—	—	33	44	32	44	69	66	
	18	—	—	—	—	42	33	72	56	50	

Table 10

Summary of Tables 8 and 9. The extents of growth are compared to those obtained in the control experiments as in Table 7. The signs denote the same as in Table 7.

	NH_4Cl	L-Glu[z]	L-Cys	DL-Try	DL-Val	Gly	DL-Thr	L-His[z]	DL-Ala	DL-Asp	DL-Met[z]	DL-Ileu[y]	Table
Str 10	os		++	s−	++	so	s		ss	so		o−	9
Str 10R	ss	++	++	so	ss	ss	so	ss	so	o−	+s		8
Str 11	os		++	o−	++	+s	s−		ss	so		+	9
Str 12	+−		++	+−	++	+s	+x		++	x−		+	9
Str 74	os		++	s	++	+o	ss		so	s−		s−	9
Str 75	+s		+	s	+	s				−			9
» »	so	++	++	+−	o−	+o	+o	xo	s−	+−	+−		8
Str 100	os	++	++	+o				os	so	s−	+o		8
» »	ss		++	so	+x	so	s		s			so	9
Str 102	−−	++	++	x−	+	ss		−−	o	x−	+−		8
Str 103	os		++	so	+s	so			so	ss		+−	9
Str 110	s−		++	so	++	+−	so		+s	s−		so	9
» »	o−	++	++	o−	s−	o−	++	++	so	x−	+o		8
Str 111	so	++	++	so	so	ss	ss	++	so	so	+o		8
Ths	so	++	++	s−		so	s−	s−	s−	so	+−		8
Str 72	so	++	++	s−	s	s−	s−	so	s−	s−	+−		8
Th	ss		++	++	++	sx	ss		s			+o	9
Str th 7952 (ATCC)	s		+	+	+				s			s	9

[z]Compound not tested in Table 9.
[y]Compound not tested in Table 8.

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